

L15 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1995:651979 CAPLUS

DOCUMENT NUMBER: 123:79976

TITLE: Efficient induction of point **mutations**  
allowing recovery of specific locus **mutations**  
in **zebrafish**

AUTHOR(S): Riley, Bruce B.; Grunwald, David J.

CORPORATE SOURCE: Dep. Human Genetics, Univ. Utah, Salt Lake City, UT,  
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SOURCE: Proceedings of the National Academy of Sciences of the  
United States of America (1995), 92(13),  
5997-6001

CODEN: PNASA6; ISSN: 0027-8424 ✓

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A technique is described that greatly increases the efficiency of recovering specific locus point **mutations** in **zebrafish** (*Danio rerio*). Founder individuals that were mosaic for point **mutations** were produced by **mutagenizing** postmeiotic gametes with the alkylating agent N-ethyl-N-nitrosourea. Under optimal conditions, each founder carried an av. of 10 **mutations** affecting genes required for embryogenesis. Moreover, .apprxeq.2% of these founders transmitted new **mutations** at any prespecified pigmentation locus. Analyses of new pigmentation **mutations** confirmed that most were likely to be point **mutations**. Thus, **mutagenesis** of postmeiotic gametes with N-ethyl-N-nitrosourea yielded frequencies of point **mutations** at specific loci that were 10- to 15-fold higher than previously achieved in **zebrafish**. Our procedure should, therefore, greatly facilitate recovery of multiple **mutant** alleles at any locus of interest.

L15 ANSWER 6 OF 6

MEDLINE on STN

DUPLICATE 3

ACCESSION NUMBER: 92331920 MEDLINE  
DOCUMENT NUMBER: 92331920 PubMed ID: 1628821  
TITLE: Induction of **mutations** in the **zebrafish**  
with ultraviolet light.  
AUTHOR: Grunwald D J; Streisinger G  
CORPORATE SOURCE: Institute of Molecular Biology, University of Oregon,  
Eugene 97403.  
CONTRACT NUMBER: GM 22731 (NIGMS)  
SOURCE: GENETICAL RESEARCH, (1992 Apr) 59 (2) 93-101. Q431.1G4  
Journal code: 0370741. ISSN: 0016-6723.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English ✓  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199208  
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AB Recessive lethal germline and specific locus somatic **mutations**  
were induced efficiently in the **zebrafish** by exposure of mature  
**sperm** to UV light. **Mutagenesis** of **sperm**  
yielded mosaic individuals: clones bearing novel **mutations**  
represented approximately 12-25% of the haploid germ cells and 25-50% of  
the somatic tissue. Simple methods are described for the reliable  
identification and propagation of newly arising developmental  
**mutations** in **zebrafish**.

L15 ANSWER 5 OF 6

MEDLINE on STN

DUPLICATE 2

ACCESSION NUMBER: 92331913 MEDLINE  
DOCUMENT NUMBER: 92331913 PubMed ID: 1628817  
TITLE: Induction of recessive lethal and specific locus  
**mutations** in the **zebrafish** with ethyl  
nitrosourea.  
AUTHOR: Grunwald D J; Streisinger G  
CORPORATE SOURCE: Institute of Molecular Biology, University of Oregon,  
Eugene 97403.  
CONTRACT NUMBER: GM 22731 (NIGMS)  
SOURCE: GENETICAL RESEARCH, (1992 Apr) 59 (2) 103-16.  
Journal code: 0370741. ISSN: 0016-6723.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199208  
ENTRY DATE: Entered STN: 19920904  
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Entered Medline: 19920818

QH431.A164

AB Recessive lethal **mutations** and **mutations** at the gol-1 locus were induced in the **zebrafish** by exposure of mature **sperm** to the alkylating agent ethyl nitrosourea (ENU). Embryonic lethal phenotypes were recognized among the parthenogenetic progeny of **mutagenized** animals or among the progeny of daughters of **mutagenized** animals. Novel specific locus **mutations** were identified by the failure of **mutagenized** chromosomes to complement pre-existing **mutant** alleles at the gol-1 locus. Each **mutagenized** individual harboured approximately 10 embryonic lethal **mutations** in its germ line and about 1 in 500 **mutagenized** animals harboured a new **mutation** at the gol-1 locus. Three lines of evidence indicate that the majority of **mutations** that were recovered following treatment of mature **sperm** with ENU were probably point **mutations**. First, the soma and germ lines of **mutagenized** animals were mosaic, as expected following simple alkylation of **sperm** DNA. Second, **mutations** induced by ENU at the gol-1 locus affected pigmentation but not viability, unlike the majority of **mutations** induced at this locus with gamma-irradiation. Third, the ratio of specific locus:recessive lethal **mutations** induced by ENU was approximately 50-fold lower than the ratio observed following **mutagenesis** with gamma-rays. Comparison of the incidence with which embryonic recessive lethal **mutations** were induced with the incidence with which specific locus **mutations** arose indicates that there are greater than 5000 genes essential to the development and viability of the **zebrafish** embryo.

L9 ANSWER 1 OF 15 MEDLINE on STN  
ACCESSION NUMBER: 95310328 MEDLINE  
DOCUMENT NUMBER: 95310328 PubMed ID: 7790347  
TITLE: Nuclear envelope breakdown is under nuclear not cytoplasmic control in sea urchin zygotes.  
AUTHOR: Sluder G; Thompson E A; Rieder C L; Miller F J  
CORPORATE SOURCE: Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545, USA.  
CONTRACT NUMBER: GM-30758 (NIGMS)  
GM-40198 (NIGMS)  
PHS-01219 (PHPPPO)  
SOURCE: JOURNAL OF CELL BIOLOGY, (1995 Jun) 129 (6) 1447-58.  
Journal code: 0375356. ISSN: 0021-9525.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199507  
ENTRY DATE: Entered STN: 19950807  
Last Updated on STN: 19960129  
Entered Medline: 19950725

QH301.J677

AB Nuclear envelope breakdown (NEB) and entry into mitosis are thought to be driven by the activation of the p34cdc2-cyclin B kinase complex or mitosis promoting factor (MPF). Checkpoint control mechanisms that monitor essential preparatory events for mitosis, such as DNA replication, are thought to prevent entry into mitosis by downregulating MPF activation until these events are completed. Thus, we were surprised to find that when pronuclear fusion in sea urchin zygotes is blocked with Colcemid, the female pronucleus consistently breaks down before the male pronucleus. This is not due to regional differences in the time of MPF activation, because pronuclei touching each other break down asynchronously to the same extent. To test whether NEB is controlled at the nuclear or cytoplasmic level, we activated the checkpoint for the completion of DNA synthesis separately in female and male pronuclei by treating either eggs or **sperm** before fertilization with **psoralen** to covalently cross-link base-paired strands of DNA. When only the maternal DNA is cross-linked, the male pronucleus breaks down first. When the **sperm** DNA is cross-linked, male pronuclear breakdown is substantially delayed relative to female pronuclear breakdown and sometimes does not occur. Inactivation of the Colcemid after female NEB in such zygotes with touching pronuclei yields a functional spindle composed of maternal chromosomes and paternal centrosomes. The intact male pronucleus remains located at one aster throughout mitosis. In other experiments, when **psoralen**-treated **sperm** nuclei, over 90% of the zygote nuclei do not break down for at least 2 h after the controls even though H1 histone kinase activity gradually rises close to, or higher than, control mitotic levels. The same is true for normal zygotes treated with aphidicolin to block DNA synthesis. From these results, we conclude that NEB in sea urchin zygotes is controlled at the nuclear, not cytoplasmic, level, and that mitotic levels of cytoplasmic MPF activity are not sufficient to drive NEB for a nucleus that is under checkpoint control. Our results also demonstrate that the checkpoint for the completion of DNA synthesis inhibits NEB by acting primarily within the nucleus, not by downregulating the activity of cytoplasmic MPF.